

Comments on the Fish Lifecycle DRP

Richard A. Becker, Ph.D., DABT
American Chemistry Council

Work Needed

- Clarify goals & objectives of the protocol to be developed as this will affect the measurement of successful validation.
- Compare efficiency and predictivity of 2-gen versus partial life cycle protocols, and consider partial life cycle (PLC) test in rainbow trout.
- Adapt protocol for use of 4 species, or substantiate empirically that fat head minnows (FHMs) are superior to other species.
- Address serious statistical concerns regarding type 1 errors, group sizes and endpoint redundancy.
- Remove redundancy and address lack of mechanistic specificity for various recommended endpoints.
- Address irrelevance of water column exposure for some chemicals and incorporate flexibility for using food route.

General Comments On The DRP

- Reproduction in a 2-gen test can be affected by non-endocrine mechanisms. The DRP should discuss the specificity of the recommended responses for endocrine mechanisms versus frank toxicity (e.g., narcosis), if known.
- Apical tests, e.g., full life cycle 2-gen or partial life cycle (PLC) tests, should incorporate a dose level targeted as a No Effect concentration.

Premature Selection of Test Species

- Focusing validation on one species - the fathead minnow (FHM) - may be more economical than validating 4 species, but a premature choice may trade short-term economy for long-term expense.
- Multigeneration and partial life cycle test periods would likely be weeks or months shorter in sheepshead minnow (SHM), medaka or zebrafish than in FHM.

Species Selection: Key Issues

- No indication that one species yields the most consistent, sensitive and specific test for hormonally active agents (HAAs)
- The longer reproductive cycle of fathead minnows may result in additional assay costs.
- The greater variability in fathead reproductive parameters likely requires larger group sizes for adequate statistical power.
- The proposed 2-gen protocol should allow use of any of the 4 species (FHM, SHM, zebrafish, medaka).

What is Gained from a Two-Gen Versus One-Gen Test?

- For non-endocrine effects, Partial Life Cycle (PLC) tests appear to be nearly as useful and more efficient than Full Life Cycle (FLC) tests, e.g. Macek & Sleight (1977); Dionne & Kiamos (1994).
 - Reported that early life stage exposure correctly predicted effects for 80% of chemicals tested.
 - Were the remaining 20% predicted within an order of magnitude dose?

PLC Versus FLC

- Partial life-cycle (e.g., OECD) versus multi-gen tests have not been rigorously compared for ability to detect HAAs or for predicting effects in the field.
- Endocrine endpoints can be routinely measured in standardized PLC protocols (e.g., OECD) and may identify HAAs as effectively and more efficiently than multigeneration tests.
- A PLC test *also* measures endpoints relevant to the population, such as the number of eggs/female/day, fertilization, hatching success, and GSI.

PLC Test in Rainbow Trout (RT) deserves consideration

- RT have a long history of regulatory use and acceptance.
- Endocrine feedback control is better understood in salmonids (e.g., RT) than in species considered in DRP.
- Synchronous reproduction strategies are important for many species (e.g., RT) but ignored in species considered in DRP.
- VTG analysis is fully developed in RT.

Statistical Considerations

- Great variability in egg production reduces the statistical power of the 2-gen test. The DRP should give greater consideration to the use of regression analysis to determine percent Effective Concentrations (e.g., EC_{20}) and incorporate such methods into the final protocol.
- The DRP should discuss the type 1 error rate expected at typical α values (e.g., 0.05 or 0.01) given that 60 - 80 NOEC determinations are recommended for the 2-gen test in FHM.

Exposure Considerations

- Exposure via the food should be considered for hydrophobic, bioaccumulative chemicals.
 - Many suspected HHAs are hydrophobic and bioaccumulative
- Maintenance of constant exposure concentrations in a long-term test is environmentally unrealistic, especially for chemicals that hydrolyze rapidly. Flexibility should be incorporated into the protocol to allow environmentally relevant exposures.

Endpoint Considerations

- Sperm quality, egg viability, and spawning behavior are redundant with hatching success or egg production - those endpoints should only be assessed if there is a specific indication that these mechanisms of action are important.
- Continuous monitoring of embryonic development is unnecessary in a 2-gen test. Significant effects on egg hatchability and larval survival would be immediately apparent.
- Sex steroid and thyroid hormone production are not useful in a FLC test; emphasis should be on reproductive results.

International Cooperation

- Must have international cooperation working through OECD EDTA
- Internationally harmonized standardized & validated methods
 - Mutual acceptance of data
 - Animal welfare